

Sediments hosting gas hydrates: oases for metazoan meiofauna

Stefan Sommer*, Erik Gutzmann, Olaf Pfannkuche

Leibniz-Institut für Meereswissenschaften, IFM-GEOMAR, Wischhofstraße 1–3, 24148 Kiel, Germany

ABSTRACT: The effect of methane seepage from sediments harbouring shallow gas hydrates on standing stocks and the distribution pattern of meiobenthic organisms, in particular Nematoda and Rotifera, was studied at about 800 m water depth at Hydrate Ridge, Cascadia subduction zone, off Oregon. The presence of shallow gas hydrates, buried only a few 10s of centimetres below the sediment surface, was indicated by extensive bacterial mats of chemosynthetic *Beggiatoa* sp. and clam fields of the bivalve mollusk *Calyptogena* spp. Mean abundances of meiobenthic organisms integrated over the upper 10 cm of the sediment were highest (1294 ind. 10 cm⁻²) at clam fields, closely followed by control sediments least affected by gas hydrates (1199 ind. 10 cm⁻²) and lowest in sediments covered with bacterial mats (762 ind. 10 cm⁻²). Average meiobenthic biomass was highest at the clam field site (262.2 µg C 10 cm⁻²), 210.4 µg C 10 cm⁻² at the control site and very low in sediments covered with bacterial mats (61.4 µg C 10 cm⁻²). The dominant taxa of meiobenthic organisms at the investigated sites were nematodes and, unexpectedly, Rotifera that are almost unknown from the deep marine habitat. In terms of abundance, rotifera dominated the meiobenthic community in gas-hydrate-influenced sediments, while control sediments and deeper basins adjoined to Hydrate Ridge were dominated by nematodes. Nematodes were concentrated in the sediment surface at all sites, whereas rotifers were almost evenly distributed at all depths, with a slight preference for deeper sediment horizons. The horizontal as well as vertical distribution of nematodes and rotifers is likely to be determined by competition or predation, and by the high adaptive capability of rotifers to highly sulphidic and anoxic conditions. Estimates of meiobenthic carbon turnover in relation to the bulk organic carbon supply indicate that, in contrast to other meiobenthic communities in cold seep environments, the meiobenthos in the studied gas-hydrate-containing sediments do not benefit from the excess availability of organic carbon via the chemoautotrophic food web. This may be because, for most meiobenthic organisms (other than rotifers), tolerance mechanisms are overwhelmed by the deleterious environmental conditions of reduced oxygen availability and extremely high sulphide fluxes.

KEY WORDS: Meiofauna · Nematoda · Rotifera · Gas hydrate · Carbon turnover · Hydrate Ridge · Cascadia subduction zone

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INTRODUCTION

Gas hydrates are solid ice-like structures in which low-molecular-weight compounds, predominantly methane, but also ethane, propane and traces of hydrogen sulphide and carbon dioxide are embedded into a lattice of water molecules (Sloan 1990). Gas hydrate deposits typically occur several 100s of metres below the seafloor. However, at Hydrate Ridge shallow gas

hydrates occur only a few decimetres below the sediment–water interface. The gas hydrates, the stability of which depends on low-temperature and high-pressure conditions, on the southern crest of Hydrate Ridge are presently within their stability field. Gas seepage is driven by dewatering processes and by chemical dissociation of gas hydrates and subsequent removal of released methane by diffusive and advective transport to the sediment surface (Suess et al. 1999). The energy

*Email: ssommer@ifm-geomar.de

bound in methane is transferred to sulphide via anaerobic methane oxidation. This process, being carried out by a consortium of methanogenic Archaea and sulphate-reducing bacteria, results in high sulphate reduction rates of up to $140 \text{ mmol m}^{-2} \text{ d}^{-1}$ in Hydrate Ridge sediments (Boetius et al. 2000). High fluxes of methane and sulphide sustain the chemosynthetically driven production of autochthonous organic carbon, which potentially enters the benthic food web and becomes available for higher eukaryotic, metazoan organisms. At seep environments, the availability of chemotrophically produced organic carbon, in addition to the input of allochthonous organic matter, is often related to increased standing stocks of specifically adapted micro-, meio- and macrobenthic communities. At Hydrate Ridge, Sahling et al. (2002) report flourishing chemosynthetically based communities of the vesicomyid clam *Calyptogena* spp. and the solemyid bivalve *Acharax* sp., which are also well documented from other seep and vent locations (cf. Olu et al. 1997, Levin et al. 2003). In the Gulf of Mexico, Fisher et al. (2000) found the new polychaete species *Hesiocaeca methanicola* colonising the surface of gas hydrates. Several studies from chemosynthetically based ecosystems such as hydrothermal vents (Kamenev et al. 1993), deep sea cold seeps (Shirayama & Ohta 1990), continental margin/shallow water cold seeps (Dando et al. 1991, 1994, Jensen et al. 1992, Buck & Barry 1998), coastal hydrocarbon seeps (Montagna & Spies 1985, Montagna et al. 1987) and brine seeps at the East Flower Garden Bank, Gulf of Mexico (Jensen 1986, Powell et al. 1986), showed that meiobenthic standing stocks benefit from the additional chemoautotrophic carbon input. From *Beggiatoa* sp. microbial mats in dysoxic and sulphidic areas in the Santa Barbara Basin, Bernhard et al. (2000) report abundant protistan and metazoan meiofaunal communities, many members of which harbour prokaryotic symbionts.

However, knowledge about metazoan meiofaunal organisms in sediments hosting gas hydrates is still very limited. Sommer et al. (2002) found elevated biomass of small-size benthic biota comprising bacteria, fungi, protozoa, and meiobenthic organisms in sediments hosting gas hydrates at Hydrate Ridge, in comparison to nearby control sites not affected by surficial gas hydrates. This correlates with the higher bacterial biomass and cell numbers at these sites described by Knittel et al. (2003). From this investigation area, Heinz et al. (2005) report slightly elevated abundances of foraminifera in comparison to control sites not affected

by surficial gas hydrates. Olu et al. (1997) found high meiobenthic abundances of up to $10\,000 \text{ ind. } 10 \text{ cm}^{-2}$ from mud volcano sediments affected by gas hydrates seawards of the Barbados accretionary prism.

In the present study, we report an apparent lack of metazoan meiobenthic response to excess availability of organic carbon in sediments strongly affected by gas hydrates. Vertical and horizontal distribution patterns of meiobenthic standing stocks and biomass, size structure, as well as estimates of meiobenthic carbon mineralisation, were examined and related to the allochthonous and autochthonous carbon input to assess the significance of gas-hydrate-derived carbon for meiobenthic carbon flow and their specific habitat demands.

MATERIALS AND METHODS

Study area and sediment sampling. Samples were collected during cruise 143/2 of RV 'Sonne' in August 1999 at 9 stations on the crest of the southern Hydrate Ridge, Cascadia subduction zone, off Oregon (Table 1). At these locations extensive seep communities associated with the occurrence of shallow methane hydrates were discovered (Suess et al. 1999, Sahling et al. 2002, Sommer et al. 2002). A detailed map and bathymetry of the sampling sites has been published by Sommer et al. (2002). Spectra of grain size, which were determined in the same subset of sediment samples used during this study, are published by Heinz et al. (2005). The gas-hydrate-affected sites at Hydrate Ridge can be divided into 3 habitats characterised by dominant key organisms that colonise along a gradient of diffusive sulphide flux (Sahling et al. 2002). Sulphide fluxes published by Sahling et al. (2002) were determined during the same cruise and at comparable stations that were sampled for the meiobenthos pre-

Table 1. Station data of RV 'Sonne' cruise 143/2, in 1999. WB: Western Basin; EB: Eastern Basin; clams and mats: with clams and bacterial mats, respectively. Dates: dd.mm

Station	Habitat	Date	Position	Depth (m)
83-1	WB	02.08	44°38.50'N, 125°14.50'W	2304
151-2	EB	14.08	44°33.00'N, 125°04.00'W	1285
91-1	Control	04.08	44°33.88'N, 125°08.29'W	852
139-1	Control	12.08	44°34.10'N, 125°08.38'W	826
190-1	Control	20.08	44°34.05'N, 125°08.41'W	824
105-1	Mats	06.08	44°34.14'N, 125°08.81'W	787
114-1	Mats	07.08	44°34.21'N, 125°09.85'W	786
187-1	Mats	19.08	44°36.16'N, 125°08.86'W	786
187-4	Mats	19.08	44°34.19'N, 125°08.82'W	785
131-1	Clams	10.08	44°34.18'N, 125°08.80'W	785
179-3	Clams	17.08	44°34.21'N, 125°08.74'W	786

sented in this study. Mats of *Beggiatoa* sp. occur directly above gas hydrates (Sites 105-1, 114-1, 187-1+4; $n = 3$) in association with high diffusive sulphide fluxes ($63 \pm 36 \text{ mmol m}^{-2} \text{ d}^{-1}$) in zones where the sulphide front reaches the surface of the sediment (Fig. 1). These bacterial mats together with the underlying sediment were sampled. Concurrently with reduced sulphide fluxes ($18 \pm 6.5 \text{ mmol m}^{-2} \text{ d}^{-1}$) and a lowering of the sulphide front several centimetres deeper into the sediment, the outer rim of the bacterial mats were densely populated by vesicomid clams of the genus *Calyptogena* spp. (Sites 131-1, 179-3; $n = 2$; Fig. 1). Sediments with lowest sulphide fluxes were characterised by the presence of the solemyid bivalve mollusc *Acharax* sp., which lives buried in the sediment. These sediments were not sampled. Control samples (Sites 91-1, 139-1, 190-1; $n = 3$) were obtained from locations without gas hydrates at a distance of a few 100s of metres from the gas-hydrate-affected sites at the southeastern slope of Hydrate Ridge. In these sedi-

ments gas hydrates were not present and dissolved sulphide was not detected. More distant reference samples were taken 10 to 15 km away from the gas hydrate sites in the Eastern (Site 151-2, $n = 1$) and Western Basins (Site 83-1, $n = 1$) flanking the Hydrate Ridge (Table 1).

A multiple corer (MUC) was used to collect almost undisturbed samples of surface sediments. The MUC was equipped with an online video/TV system to allow controlled sampling of specific spots on the seafloor. The MUC retrieved up to eight 10 cm diameter sediment cores of a maximal length of 35 cm. Upon recovery, the cores were immediately transferred to a 4°C cold room for further processing. From each MUC cast 1 single core was selected for meiofaunal analysis. Selection criteria for these cores from the respective MUC casts were the presence of clams or microbial mats and the quality of the sediment sample such as undisturbed and even sediment surface. At reference and control sites cores were selected based on the quality of the

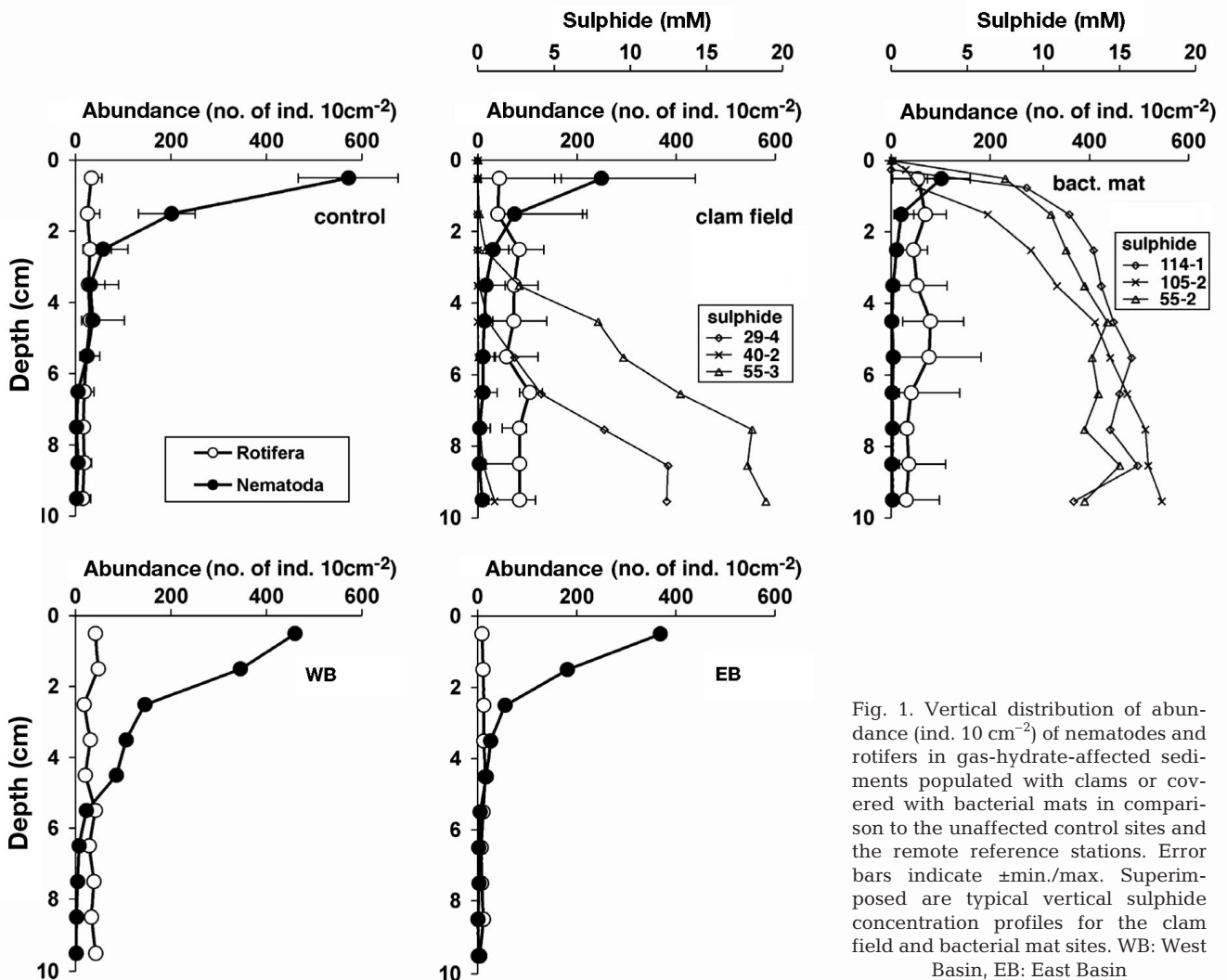


Fig. 1. Vertical distribution of abundance (ind. 10 cm⁻²) of nematodes and rotifers in gas-hydrate-affected sediments populated with clams or covered with bacterial mats in comparison to the unaffected control sites and the remote reference stations. Error bars indicate ±min./max. Superimposed are typical vertical sulphide concentration profiles for the clam field and bacterial mat sites. WB: West Basin, EB: East Basin

sample. Each selected MUC core was subsampled twice with cut off syringes of 2.1 cm diameter. Subsamples were sectioned horizontally in 1 cm intervals down to a depth of 10 cm. Both subsamples from the respective depth horizons were pooled and preserved in 10% formalin buffered with Borax (pH = 8.2).

Meiofaunal analysis. In the laboratory meiofaunal organisms were extracted employing the flotation technique (Pfannkuche & Thiel 1988) using LUDOX AM. The extraction procedure was repeated twice yielding an extraction efficiency of >80%. Meiobenthic organisms were concentrated on a 32 µm sieve, preserved in 10% formalin and stained with Rose Bengal. Meiofaunal organisms were identified on a gross taxonomical level and were enumerated in a Bogorov tray using a Leica MZ 8 dissection microscope at a magnification of ×16. Nematode biomass was determined morphometrically according to Andrassy (1956). For the determination of the remaining meiobenthic biomass, the organisms were transferred into tin cups and dried at 60°C for 48 h. Carbon and nitrogen contents were determined using a Carlo Erba protein analyser NA 1500. For calibration, an acetanilide standard (C₈H₉NO; 71.09% C, 10.36% N) and a certified soil standard BSTD 1 (3.5% C, 0.216% N) were used. Due to the small size of rotifers and difficulties in subsampling for C/N determination, these organisms were not included into biomass calculations but biomass of rotifers might be considered negligible.

Sulphide measurements. After recovery, the retrieved sediment cores were rapidly transferred to the onboard laboratory, which was cooled to 4°C. The cores were segmented into 1 cm slices for pressure fil-

tration. Porewater was squeezed from the sediment through 0.2 µm cellulose acetate membrane filters at up to 3 bar pressure, applying argon gas with a mechanical polypropylene press. Sulphide concentrations in the porewaters were measured on board using a standard photometric procedure following Grasshoff et al. (1983). Due to strong spatial habitat heterogeneity and the amount of sediment needed to retrieve sufficient porewater, sulphide concentration profiles were not determined in the same sediment cores, which were used for meiofaunal analyses or in cores retrieved from the same MUC cast but in comparable habitats. Comparability is based on the presence or absence of the key organisms *Beggiatoa* sp. and *Calyptogenia* sp.

RESULTS

Composition of meiobenthos, abundance, biomass and size

Nematodes (13 to 90%) and rotifers (4 to 85%) dominated all sites, followed by copepods and polychaetes. Members of other taxa occurred only irregularly and represented minor constituents of the meiobenthos (Table 2). At the control sites and in the adjacent basins, nematodes dominated the meiofauna. However, with increasing sulphide concentrations, rotifers became the dominant taxon, at average representing 49 and 74% of the meiobenthic community in clam fields and sediments covered by bacterial mats, respectively. Copepods were less successful, the highest dominance of 7% was found in the Eastern Basin;

Table 2. Depth-integrated abundances (ind. 10 cm⁻²) at the gas-hydrate-affected sites of clam fields (179-3, 131-1) and microbial mat systems (114-1, 187-1+4, 105-1) in comparison to control (91-1, 139-1, 190-1) and reference stations (WB: Western Basin [83-1]; EB: Eastern Basin [151-2])

Taxa	WB	EB	Controls			Clam fields		Bacterial mats		
	83-1	151-2	91-1	139-1	190-1	179-3	131-1	114-1	187-1+4	105-1
Nematoda	1180	661	811	943	1011	626	467	213	80	195
Rotifera	343	102	40	338	194	887	429	417	529	736
Kinorhyncha	5	3	1	4	3	4	0	0	0	0
Gastrotricha	1	3	0	0	0	0	0	0	0	0
Priapulida	4	0	0	0	0	0	0	0	0	0
Tardigrada	0	0	1	0	0	0	0	0	0	0
Copepoda	39	66	20	38	27	1	48	1	0	1
Ostracoda	7	0	1	1	1	10	4	1	4	6
Amphipoda	1	1	0	0	0	0	6	1	1	0
Tanaidacea	0	0	0	0	1	0	0	0	0	0
(Nauplii)	54	73	11	57	27	26	57	4	6	10
Polychaeta	14	19	15	22	18	12	9	48	0	13
Oligochaeta	1	1	5	1	0	0	1	0	3	0
Gastropoda	0	0	0	0	1	0	0	10	0	1
Bivalvia	0	1	0	1	3	0	0	2	0	3
Total	1649	930	905	1405	1286	1566	1021	697	623	965
Mean	1649	930		1199		1294		762		

in bacterial mat sediments, they represented <1% of the total meiobenthos. The results below will be described with major focus on the sites atop Hydrate Ridge; data from the adjoining basins, where no replicate sampling has been conducted, will only be used as background information.

Total meiofaunal abundance (integrated over the upper 10 cm sediment) at Hydrate Ridge was highest at the clam field site (1294 ind. 10 cm⁻²); in bacterial mat sediments, abundance was lowest (762 ind. 10 cm⁻²; Table 2). In the control sediments, abundance was slightly lower than in the clam beds and varied between 905 and 1405 ind. 10 cm⁻².

Total meiobenthic biomass (integrated over the upper 10 cm sediment) ranged between 61.4 µg C 10 cm⁻² at bacterial mats and 262.2 µg C 10 cm⁻² at clam field sites (Table 3). Due to the small size of the rotifers, which were only 70 µm in length, and difficulties in sampling them for C/N analysis, they were not considered in the total meiofaunal biomass. Comparison of the total meiobenthic biomass between the different sites needs careful evaluation due the limited data set and the heterogeneity of the habitats. Furthermore, the stochastic occurrence of single meiobenthic organisms with high biomass can introduce strong variability into the measurement of the total meiobenthic biomass. Despite this, the vertical distribution patterns of the total meiobenthic biomass indicate very low biomass at the mat sites compared to the clam field and control sites (Fig. 2). Between the sediments populated with clams and the control sites, no distinct difference in total biomass can be revealed.

The maximum mean nematode population biomass (integrated over the upper 10 cm sediment) was found in the control sediments reaching 62.4 µg C 10 cm⁻². In sediments covered with clams and microbial mats, average nematode population biomass was about 1.5 and even 8.2 times lower, respectively (Table 3). Nematode population biomass in the Eastern Basin, where we found the sulphide-oxidising bacteria *Thioploca* sp., was similar to that of the bacterial mat site,

whereas, in the deeper Western Basin, it was similar to that of the clam field sites. At the gas-hydrate-affected sites of clam fields and microbial mat sediments, the relative contribution of the nematode population biomass to the total meiofaunal biomass was low and varied between 16 and 14%, respectively, in contrast to 30% at the control sites. Individual nematode biomass varied strongly between 0.018 µg C in the Eastern Basin and 0.083 µg C in clam field sediments (Table 3). In comparison to the microbial mat and control sediments, highest average individual nematode biomass, coinciding with the highest average length, was found at the clam fields (Table 3). In the deeper Eastern and Western Basins, nematodes were on average 123 and 304 µm shorter compared to the control site, and followed the generally observed trend of decreasing length with increasing depth.

Vertical distribution patterns of abundance, biomass and size

At all investigated sites, nematodes showed distinct surface maxima (Fig. 1). The penetration depth of nematodes in microbial mat sediments, where high sulphide concentrations were present close to the surface of the sediment, was strongly confined to the uppermost, 2 cm thick sediment layer. Although single filaments of the motile *Beggiatoa* sp. were observed deeper in the sediment, their depth distribution was predominantly restricted to the surface of the sediment. The abundance of nematodes in this uppermost sediment layer was greatly reduced in comparison to the corresponding sediment horizons in the control sediments and clam fields. In clam field sediments, where the onset of increased sulphide concentrations was typically measured at a sediment depth of 2 to 4 cm, and, in control sediments, containing no dissolved sulphide in the porewater, nematodes penetrated deeper into the sediment. Although at the bacterial mat and clam field sites the variability of rotifer

Table 3. Mean total meiofaunal biomass (min.–max.), individual nematode biomass (±SD), mean nematode population biomass (min.–max.), as well as the mean lengths (±SD) and widths (±SD) of nematodes in the different habitats (WB: Western Basin; EB: Eastern Basin)

	WB	EB	Controls	With clams	With bacterial mats
Total meiofauna					
Biomass (µg C 10 cm ⁻²)	181.3	363.5	210.4 (86–335)	262.2 (166–359)	61.4 (20–120)
Nematoda					
No. of nematodes measured	487	658	1200	371	228
Ind. biomass (µg C)	0.047 ± 0.085	0.018 ± 0.030	0.072 ± 0.017	0.083 ± 0.009	0.050 ± 0.020
Pop. biomass (µg C 10 cm ⁻²)	45.9	10.8	62.4 (45–75)	42.6 (41–44)	7.6 (1–14)
Mean length (µm)	690 ± 465	509 ± 331	813 ± 65	1269 ± 276	848 ± 165
Mean width (µm)	25 ± 11	19 ± 8	28 ± 13	27 ± 11	22 ± 10

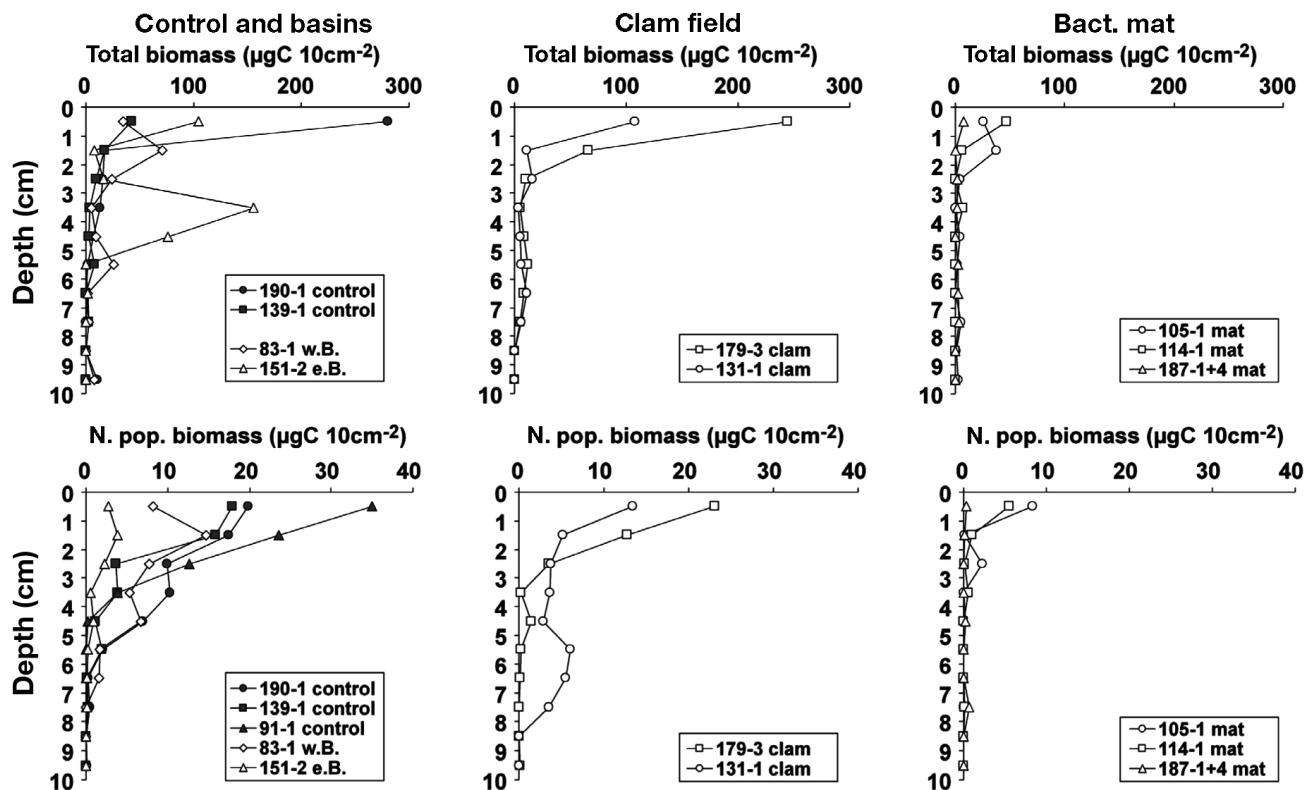


Fig. 2. Vertical distribution of total meiobenthic biomass ($\mu\text{g C } 10\text{ cm}^{-2}$, excluding rotifers) and nematode population (N. pop.) biomass ($\mu\text{g C } 10\text{ cm}^{-2}$) in gas-hydrate-affected sediments populated with clams or covered with bacterial mats in comparison to the unaffected control sites and the remote Western and Eastern Basin (w.B. and e.B., respectively) reference stations

distribution was very high, rotifers tended to colonise deeper sediment horizons under highly sulphidic conditions (Fig. 1). In the Western and Eastern Basins, nematodes also showed distinct surface maxima but penetrated deeper into the sediment. The distribution of rotifers was almost uniform, with a slight tendency towards an increase of abundance with depth.

At all sites atop Hydrate Ridge, the meiobenthic biomass was concentrated at the surface of the sediment (Fig. 2). In sediments of the Western and Eastern Basins, elevated meiobenthic biomass was also found in deeper horizons. Nematode population biomass showed distinct surface maxima at the gas-hydrate-affected sites. At the clam field site (131-1) elevated biomass was found at a depth interval of 5 to 6 cm, coinciding with a maximum in nematode length (Figs. 2 & 3). At the control sites, with no sulphide present in the porewater, the nematode population biomass gradually decreased with depth. At the Western and Eastern Basins, nematode population biomass displayed a subsurface maximum at 1 to 2 cm depth and decreased gradually with depth.

In control sediments the vertical distribution of nematode lengths showed a unimodal distribution, with a maximum mean length of 1322 μm at 3 to 4 cm depth (Fig. 3). At the clam field sites, the distribution of

nematode lengths appeared to be bimodal, with a maximum mean length of 1600 μm at 2 to 3 cm and a second maximum of 2059 μm length at 5 to 6 cm depth. These high length values were caused by the presence of nematodes belonging to the genus *Metalinhomoeus* sp. de Man, 1907. The shortest nematodes were encountered at a sediment depth of 3 to 4 cm, coinciding with the vertical position of the sulphide front at the clam field sites. In sediments covered with bacterial mats, a pronounced maximum of 2057 μm , similar to the average length found in clam fields at 5 to 6 cm sediment depth, occurred at a sediment depth of 1 to 2 cm. In the Western and Eastern Basins the average length of nematodes steadily increased with depth.

DISCUSSION

Meiobenthic response to excess availability of organic carbon

At the gas-hydrate-affected sites at Hydrate Ridge chemosynthesis provides excess supply of autochthonous organic carbon in addition to the carbon input through the water column (allochthonous carbon source). The relative proportion of this autochthonous

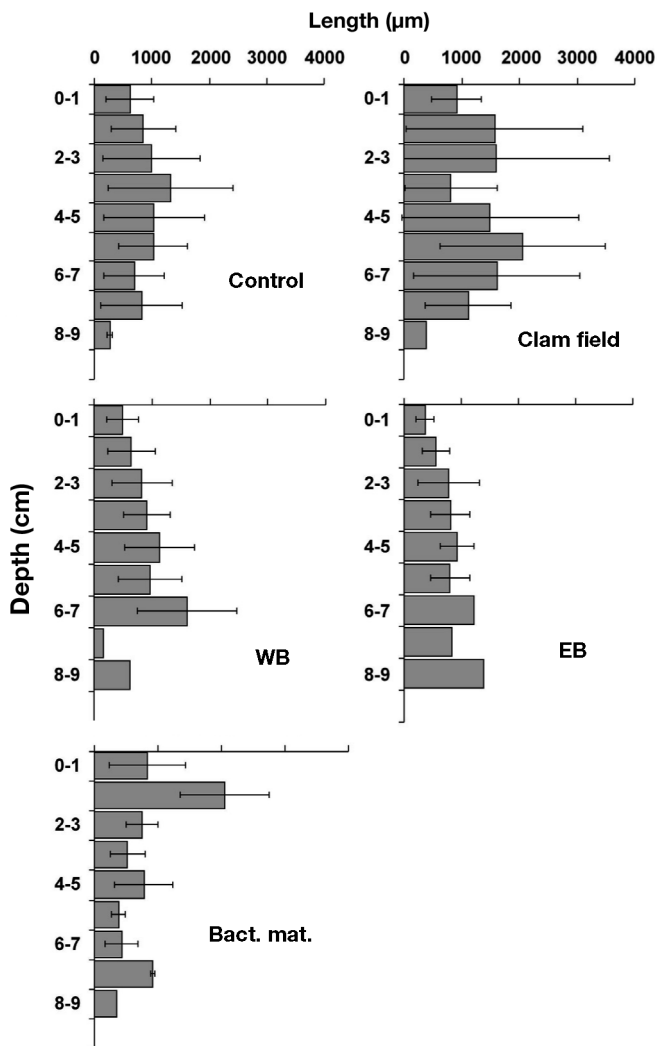


Fig. 3. Vertical distribution of mean nematode length (μm) in gas-hydrate-affected sediments populated with clams or covered with bacterial mats in comparison to the unaffected control sites and the remote reference stations. Error bars: SD. WB: West Basin, EB: East Basin

organic carbon production from the bulk particulate organic carbon (sum of allochthonous and sedimentary autochthonous autotrophic particulate organic carbon, POC) available for the benthic food web is about 75% at the clam field sites and 52% in sediments covered with bacterial mats, in contrast to only 11% at the control sites (Sommer et al. 2002). As indicated by the elevated biomass of the small-sized benthic biota (Sommer et al. 2002), increased microbial biomass and total cell numbers of bacteria (Knittel et al. 2003), and distinct macrobenthic communities (Sahling et al. 2002) at our investigation area, one might expect that this excess supply of organic carbon potentially contributes towards higher standing stocks and productivity of meiobenthic organisms. Analyses of foraminiferal

assemblages, which were conducted on the same subset of sediment samples used during this study, revealed a trend towards higher standing stocks in gas-hydrate-affected sites (Heinz et al. 2005). However, although there are higher meiofaunal abundances in the sediments populated by clams, in terms of biomass, the total meiofauna and especially nematodes at these gas-hydrate-affected sites apparently did not respond to this excess supply of autochthonous organic carbon. To estimate the relative proportion of meiobenthos in the carbon flow of these seep systems, meiobenthic carbon demand (integrated over the upper 10 cm of the sediment) has been calculated for the different sites except the Western and Eastern Basins (Table 4). We used the allometric equation of Mahaut et al. (1995), which relates the individual respiration rate R (d^{-1}) to the mean individual weight W (mg C) of meiofaunal organisms. However, these calculations might only reveal general trends. Seep organisms might have specific physiological adaptations with respect to respiration which are not accounted for by Mahaut et al.'s (1995) approach.

Meiofaunal carbon demand declines from $12.0 \text{ mg C m}^{-2} \text{ d}^{-1}$ at control sites to $2.5 \text{ mg C m}^{-2} \text{ d}^{-1}$ in sediments covered with bacterial mats characterised by highest sulphide fluxes (Table 4). At the microbial mat sites, the relative proportion of total meiobenthic carbon demand represents only 1.5 to 2.8% from the bulk organic carbon input. Using a molar ratio between oxygen consumption and POC degradation of 1.4 (Anderson & Sarmiento 1994), an oxygen consumption of the meiobenthic community of $0.1 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ can be calculated for microbial mat sites. This corresponds to 0.2% of the total oxygen uptake ($47.5 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$), which was measured at this site under *in situ* conditions with benthic landers (Sommer et al. 2006). In clam field sediments, the input of autochthonous organic carbon was 3.2-fold higher than at microbial mat sites. This might be related to the 4 times higher total meiofaunal biomass or the 6-fold higher nematode biomass. However, despite this increase of meiofaunal standing stocks in comparison to the microbial mat sites, meiofaunal carbon mineralisation ($11.7 \text{ mg C m}^{-2} \text{ d}^{-1}$) represents only 3.9 to 5.4% of the bulk organic carbon input (Table 4). Meiofaunal oxygen consumption ($0.5 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$) at the clam field sites corresponds to 14% of the total oxygen uptake ($3.7 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$; Sommer et al. 2006). The highest carbon demand was found at the control sites, where allochthonous organic matter imported through the water column was the major carbon source. At these sites the relative proportion of meiofaunal mineralisation from the bulk organic carbon flux ranged between 10.7 and 36.6%. Meiofaunal oxygen consumption represents about 25% of the total oxygen

Table 4. Meiobenthic carbon turnover at the gas-hydrate-affected sites in comparison to the control sites. Respiration rates have been calculated using the allometric equation, relating the individual respiration rate R (d^{-1}) to individual weight W (mg C): $R = 7.4 \times 10^{-3} \times W^{-0.24}$ (Mahaut et al. 1995). Estimates of the bulk particulate organic carbon input available for benthic consumers, defined as the sum of the allochthonous carbon input imported through the water column and the autochthonous carbon input introduced via chemosynthetic processes, were provided from Sommer et al. (2002)

Measure	Control sites	Clam fields	Microbial mats
Bulk C_{org} input ($\text{mg C m}^{-2} \text{d}^{-1}$)	32.8–111.9	218.3–300.3	88.0–170.0
Autochthonous C_{org} input ($\text{mg C m}^{-2} \text{d}^{-1}$)	5.7	190.0	59.7
Mean respiration rate (d^{-1})			
Nematoda	0.073	0.071	0.080
Remaining meiofauna	0.034	0.033	0.035
Carbon demand ($\text{mg C m}^{-2} \text{d}^{-1}$)			
Nematoda	4.9	3.0	0.6
Remaining meiofauna	7.1	8.7	1.9
Total meiofauna	12.0	11.7	2.5
Rel. prop. from bulk C_{org} input (%)	10.7–36.6	3.9–5.4	1.5–2.8

uptake. Although differentiation between the different carbon sources is not possible, the meiofaunal carbon demand in clam field sediments and at microbial sites could be easily covered by the input of allochthonous organic matter (27.1 to 110.3 $\text{mg C m}^{-2} \text{d}^{-1}$; Sommer et al. 2002).

When moving from the control sites and clam fields to microbial mats, the sediments became more biogeochemically active in terms of increasing fluxes of methane, sulphate, sulphide, total uptake of oxygen and anaerobic turnover of methane (Sahling et al. 2002, Sommer et al. 2006), but the carbon turnover of the metazoan meiobenthos declined drastically. Reduced availability of oxygen, high fluxes of sulphide that are toxic for all aerobically living metazoans by blocking the cytochrome-*c*-oxidase of their respiratory chain (Bagarinao 1992), and food quality might be considered as possible reasons for an insignificant meiobenthic response towards the excess supply of organic carbon at the gas-hydrate-affected sites.

Meiobenthos at microbial mat sites

There is still a lively debate on the effects of lack of oxygen on the distribution and structure of meiobenthic communities; often they are difficult to separate from other environmental factors such as food availability and occurrence of high-sulphide porewater concentrations in anoxic environments. In shallow-water habitats with extended periods of anoxia in the overlying water body (Hendelberg & Jensen 1993), meiobenthic animals, particularly nematodes, have been found to be extremely tolerant of low oxygen

concentrations. Respiration measurements and experiments on the tolerance towards low oxygen concentrations (Ott & Schiemer 1973, Schiemer et al. 1990) demonstrated that even species which are found in anoxic sediments use oxygen when it becomes available. Based on theoretical considerations and oxygen consumption rates of different meiobenthic groups, Powell (1989) concluded that aerobic metabolism can be maintained at oxygen concentrations as low as $0.1 \mu\text{mol l}^{-1}$, which is below the detection level of present methods for the determination of oxygen.

At the studied bacterial mat sites, oxygen availability for meiofaunal respiration is severely restricted. Filamentous sulphide-oxidising bacteria of the genus *Beggiatoa* sp. position themselves within a steep gradient of oxygen and sulphide, tolerating only very low amounts of both (Jørgensen 1987). Oxygen penetration in microbial mats at Hydrate Ridge is less than a few millimetres (Knittel et al. 2003). Total oxygen uptake of the microbial mat sediments at Hydrate Ridge was extremely fast, with rates of up to $53.3 \text{ mmol m}^{-2} \text{d}^{-1}$ (Sommer et al. 2006). Mass balance calculations for these sites indicate that most of the oxygen was used by the sulphide-oxidising bacteria *Beggiatoa* sp., probably out-competing oxygen uptake of infaunal eukaryotic organisms. Below the oxygen penetration depth, sediments become highly reducing, with sulphide concentrations of up to 18 mmol l^{-1} in the upper 10 cm of the sediment. Thus, the availability of trace amounts of oxygen deeper in the sediment can be almost excluded. This is also consistent with the very low penetration depth of nematodes at these sites. Hydrate Ridge is located within the northern outreach of the extensive California continental margin oxygen minimum zone. This, in addition to the specific biogeochemical conditions caused by the presence of gas hydrates and high concentrations of methane and sulphide, might induce further effects on the benthic community structure and productivity. Oxygen in the bottom water, measured about 30 cm above the sediment–water interface, varied between 32.6 and $45.1 \mu\text{mol l}^{-1}$ (Sommer et al. 2006). From Volcano 7 projecting into an oxygen minimum zone, Levin et al. (1991) report meiobenthic abundances of $194 \times 10^3 \text{ ind. m}^{-2}$ atop the volcano, which is 3.6 times lower than the maximum abundances recorded in this study. The top of Volcano 7 is located at about the same water depth (730 to 770 m) as Hydrate Ridge and is

characterised by extremely low bottom water oxygen concentrations ($<4.5 \mu\text{mol l}^{-1}$). From an oxygen minimum zone 700 m deep at the continental slope of Oman, Cook et al. (2000) report 12.8 times higher nematode abundances of $2495 \times 10^3 \text{ ind. m}^{-2}$ than those found during this study. Levin et al. (1991) and Cook et al. (2000) relate their findings of high meiobenthic abundances in oxygen minimum zones to a high availability of allochthonous organic carbon.

In contrast to our findings, high meiofaunal abundances of $1.9 \times 10^6 \text{ ind. m}^{-2}$ were found at coastal hydrocarbon seeps associated with mats of *Beggiatoa* sp. (Isla Vista, California) (Montagna & Spies 1985), and higher standing stocks were reported from inside bacterial mats in comparison to outside (Montagna et al. 1987). Higher meiobenthic stocks at these seeps might be due to the additional carbon input provided by the seeping oil. Oil seepage does not take place at Hydrate Ridge gas-hydrate-affected sites. Furthermore, sulphide concentrations in Hydrate Ridge sediments covered with microbial mats are extremely high and almost unparalleled by concentrations reported from other meiobenthic studies in extreme environments. Meiofaunal organisms have developed several strategies and detoxification mechanisms to withstand sulphidic conditions (cf. Fenchel & Finlay 1995); however, some of them depend on access to oxygen, at least temporarily. From the nematode species *Oncholaimus campylocercoides* living at shallow-water hydrothermal vent sites off the Greek Island Milos, Thiermann et al. (2000) reported that its sulphide tolerance under hypoxic conditions became greatly reduced when ambient sulphide levels reached 1 mmol l^{-1} . Powell (1989) and Jahn et al. (1997) calculated that, depending on the ambient sulphide concentration, diffusive sulphide flux into the body tissue of small-sized organisms can be very fast, literally flooding them and overriding any detoxification mechanisms. We propose that, in the Hydrate Ridge microbial mat sediments overlying shallow gas hydrates, the adaptive capability of nematodes is exhausted due to extremely high sulphide concentrations coupled with limited oxygen availability.

Astonishingly, in microbial mat sediments, we found rotifers belonging to the genera *Monostyla* sp. and *Lecane* sp. (Sommer et al. 2003) dominating the meiofauna in terms of abundance. Although in lower abundances, these organisms also represented a prominent component of the meiobenthic community in the sediments of the Western and Eastern Basins, indicating that these organisms are not specific seep inhabitants. Their vertical distribution indicates that rotifers avoid colonising surface horizons where nematodes dominate. Rotifers are among the smallest metazoan organisms (diameter: $\sim 40 \mu\text{m}$; length: $\sim 70 \mu\text{m}$); thus, they

probably contribute to the food of large predatory and omnivorous nematodes, restricting their distribution. We do not know how these organisms cope with the extremely high sulphidic conditions in deeper sediment layers. It remains speculative whether these rotifers are true anaerobes, which would reduce sulphide toxicity. However, the high variability of their vertical distribution is probably related to enhanced vertical migration between oxygenated surface horizons and deeper zones to escape predation.

Beside oxygen availability and presence of dissolved sulphide, the vertical distribution of bulk meiobenthic abundance and biomass is apparently related to the availability and turnover of allochthonous and autochthonous organic carbon. The vertical distribution of these carbon sources, deduced from vertical turnover profiles of $^{14}\text{CO}_2$ uptake (chemosynthesis), turnover of fluorescein-di-acetate (FDA) and plant pigments is confined to the uppermost 3 cm of the sediment (Sommer et al. 2002). FDA indicates the potential activity of some extracellular hydrolytic enzymes involved in the degradation of organic matter. These parameters were determined during the same cruise and at the same sites that were sampled for meiobenthos in the present study. The comparison of size distributions of nematodes showed that at these depth horizons (1 to 2 cm sediment depth) longer nematodes prevail, with an average length of $2057 \mu\text{m}$. This finding points towards an altered species composition of the nematode community in comparison to the other depth horizons in which nematode length is generally $<1000 \mu\text{m}$. In sulphidic, shallow-water habitats, Jensen (1987) identified such longer and more slender nematodes as thio-biotic species, being adapted to sulphidic and anoxic conditions.

Meiobenthos at clam field sites

Elevated meiofaunal standing stocks are likely to be due to the improved environmental conditions caused by the pumping activity of overlying seawater through the mantle cavity of *Calypptogena* spp. This depresses the sulphide front several centimetres into the sediment (Sahling et al. 2002, cf. Fig. 1) and leads to a relocation of solutes and micro-particulates deeper into the sediment. Vertical distribution of bacterial cell counts, microbial activity (Knittel et al. 2003), modelling of geochemical porewater profiles (Luff & Wallmann 2003) and distribution of plant pigments (Sommer et al. 2002) confirm the importance of this transport mechanism at these sites. For clam fields at mud volcanoes seaward of the Barbados accretionary prism, Olu et al. (1997) described density-driven convectional fluid flow induced by gas-hydrate dissolution. Similarly, at cold

seeps in the Kattegat (Dando et al. 1994), fluid seepage induced interstitial water circulation associated with a draw-down of overlying seawater into the sediment close to the seeps. These authors concluded that convective transport processes enhance interfacial fluxes of solutes and micro-particulates, which might create micro-niches of high microbial activity. Such microbially highly active zones have also been found in the vicinity of burrow walls, due to enhanced influx of solutes induced by macrofaunal bioirrigation. Wetzel et al. (1995) found that such zones around the burrow walls of the lugworm *Arenicola marina* are highly attractive to meiofaunal organisms. These processes act on small spatial scales (millimetres to centimetres), which render the sampling of such habitats very difficult, and they might contribute to the variability of meiobenthic distribution patterns as has been observed during this study.

The biomass maximum at the surface of the sediment is related to the availability of allochthonous organic carbon as indicated by vertical concentration profiles of chlorophyll *a* and phaeopigments (Sommer et al. 2002). The slightly elevated biomasses 5 to 7 cm deep in the sediment might be related to a pronounced peak of the chemotrophic $^{14}\text{CO}_2$ uptake in a depth of 5 cm, indicating the production of additional endogenous organic carbon available to the benthic food web (Sommer et al. 2002). This maximum further coincides with an increased turnover of FDA. The mean length of nematodes in clam fields is significantly greater than at the microbial mats. This indicates an overall altered species composition of the nematode community in comparison to the other sites. The high average length at the clam field site is due to the presence of nematodes belonging to the genus *Metalinhomoeus* sp., which represents a prominent component of nematode communities in other organically enriched and sulphidic habitats (Dando et al. 1991, Wetzel et al. 1995). Although size distribution of nematodes at clam fields points towards an adaptive capability of the nematode community, nematode population biomass and abundance at this site are still lower than at the control site. As observed in microbial mats, nematodes in clam field sediments still might suffer from severe environmental conditions. Oxygen, when available, is needed for the maintenance of aerobic metabolism, chemosynthesis and chemical oxidation processes; however, a certain part is also likely to be required to support sulphide detoxification. Nematodes might experience longer periods of anaerobiosis, which energetically is not favourable and results in low productivity. Other reasons for the lower abundance and population biomass of nematodes might include mining activities of the clams (i.e. using their feet to obtain sulphide), and the quality of food.

Meiobenthos in the basins

At the more distant and deeper reference site in the Western Basin, meiobenthic biomass and abundance were similar to those determined at the control sites. Due to the proximity of the Western Basin to the Hydrate Ridge and the lateral organic carbon input, this might be related to an enhanced supply of allochthonous organic carbon, as indicated by similar inventories of plant pigments (Sommer et al. 2002). Although no sulphide has been detected in the upper 10 cm of the sediments, in the Western and Eastern Basins, a slightly higher autochthonous carbon input has been determined (Sommer et al. 2002). This additional input of organic carbon coincides with the observation of the sheath-building bacteria *Thioploca* sp. in our sediment samples. *Thioploca* sp., a close relative of *Beggiatoa* sp., is a facultative chemolithoautotroph, able to gain energy from the oxidation of sulphide with oxygen or nitrate. Despite high sulphate reduction rates, surface sediments harbouring *Thioploca* sp. are generally characterised by low concentrations of sulphide, which might further promote meiobenthic colonisation, potentially exploiting the excess supply of organic carbon.

Acknowledgements. We are grateful for the support of the officers and crew of RV 'Sonne' during Cruise SO No. 143/2. We thank A. Kähler for taking and preserving meiofaunal samples, B. Domeyer for the sulphide measurements, W. Queisser for his technical assistance on board and K. Georgetleit for editing the manuscript. The SO No. 143/2 cruise was supported by the German Federal Ministry of Research and Education (BMBF) as part of the TECFLUX project, Contract No. Fkz03G0143A. This is Publication No. GEOTECH-179 of the programme Geotechnologien of BMBF (Bundesministerium für Bildung und Forschung) and DFG (Deutsche Forschungsgemeinschaft).

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